***Crinalium epipsammum* sp. nov.: a filamentous cyanobacterium with trichomes composed of elliptical cells and containing poly- β -(1,4) glucan (cellulose)**

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This paper describes the isolation and characterization of a new species of cyanobacterium, *Crinalium epipsammum*. The assignment to the genus *Crinalium*, first described by Crow (1927), is based on a property which is unusual for cyanobacteria: trichomes viewed in cross-section are elliptical rather than circular. This organism was isolated from the surface layer of sandy soil of coastal dunes in The Netherlands. The organism is non-motile and drought resistant, and its cell surface is hydrophilic. The temperature optimum for growth is 25 °C. The mean DNA base composition is 33.9 mol% G + C. The cell wall is relatively thick and contains poly- β -(1,4)glucan (cellulose), which is unusual for cyanobacteria.

Introduction

Cyanobacteria are oxygenic phototrophic organisms. They are often found in nutrient-depleted environments and may represent the dominant population under extreme environmental conditions. Cyanobacteria are also known to form crusts in desert or tropical soils (Roger & Reynaud, 1982). In such environments they thrive under conditions of low matrix water potential. Recently, we characterized the crusts of the coastal dunes of the North Sea in The Netherlands (De Winder *et al.*, 1989), which generally consist of the eukaryotic green alga *Klebsormidium flaccidum* and a few species of cyanobacteria (e.g. *Microcoleus* spp. and *Oscillatoria* spp.). However, studies on population dynamics showed the frequent dominance of an unusual filamentous cyanobacterium with elliptical cells during early stages of crust formation (De Winder *et al.*, 1989).

The colonization of dune sand by cyanobacteria and green algae is considered to play an important role in protection against erosion (Van den Ancker *et al.*, 1985). Since this environment is exposed to long periods of drought, it is clear that the inhabiting organisms must be drought tolerant. To study the specific adaptations of the crust-building phototrophic organisms with respect to

drought resistance, the dominant algae and cyanobacteria were isolated. This paper reports the isolation and characterization of the filamentous cyanobacterium whose trichomes are composed of elliptical cells. The isolate was assigned to the genus *Crinalium*, first described by Crow (1927), but of which cultured representatives were previously lacking (Anagnostidis & Komarek, 1988; Castenholz, 1989). The new species *Crinalium epipsammum* is proposed for this organism.

Methods

Isolation and growth conditions. *Crinalium epipsammum* was isolated from microbial crusts, collected from so-called 'blow outs' (aeolian erosional depressions) on south-exposed slopes of sand dunes (Jungerius *et al.*, 1981) in a coastal dune area on the Dutch North Sea coast, which were especially enriched at the edges and centre with this organism. Crust material was suspended in medium BG-11 (Rippka *et al.*, 1979) and incubated at 20 °C under continuous illumination with a photon flux density (PFD) of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After 5 d the enrichment cultures were plated on BG-11 medium solidified with 1% (w/v) agar (Difco Bacto). *C. epipsammum* was isolated and purified by micromanipulation. Continuous and exponentially growing batch cultures were slightly contaminated with bacteria, but the degree of contamination never exceeded 1% on the basis of cell number (as judged by microscopic examination). The strain has been deposited at the Culture Collection of Algae of the Institute of Plant Physiology of the University of Göttingen, Federal Republic of Germany (strain no. SAG 22.89).

Cultures were maintained at 20 °C under continuous illumination with white fluorescent lamps (Philips TLE 32/33) at a PFD of

Abbreviations: DAP, diaminopimelic acid; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; DMF, dimethylformamide; PFD, photon flux density; SAG, Sammlung von Algenkulturen Göttingen.

$10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and were continuously sparged with air (approximately 40 l h^{-1}). Experiments were performed with two types of cultures: (i) batch cultures, grown at 20°C and pH 8.0–8.5 under continuous light at a PFD of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$, continuously sparged with air at approximately 40 l h^{-1} (growth rate 0.029 h^{-1}); (ii) continuous cultures ($D = 0.011 \text{ h}^{-1}$), grown at 20°C and pH 8.1 in a light:dark cycle of 8:16 h, at an average PFD of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$. Cells from batch cultures were harvested at $\text{OD}_{750} = 0.5$ ($52.5 \pm 2.5 \text{ mg protein l}^{-1}$), corresponding to the late exponential phase of growth. Continuous cultures were sampled in steady state. Biomass was measured as protein content or OD_{750} . Growth at different salinities was determined by using full-strength BG-11 medium; ten-, five- and two-fold diluted BG-11; and BG-11 with 20, 100 and 200 mM-NaCl .

Nitrogen fixation. Nitrogenase activity was investigated by following the experimental protocol of Rippka & Waterbury (1977). Batch cultures were washed three times in nitrate-free BG-11 medium. After resuspension in the latter medium, the cells were starved for combined nitrogen for 48 h under a gas phase of air (40 l h^{-1}) and at a PFD of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$. The cell suspension was then concentrated tenfold, and DCMU was added to a final concentration of 10^{-5} M to inhibit oxygenic photosynthesis. After flushing with Ar , the anaerobic suspension was incubated in the light (PFD $20 \mu\text{mol m}^{-2} \text{s}^{-1}$) and nitrogenase activity was monitored every hour for the first 10 h of incubation and once after 24 and 48 h by measuring acetylene reduction (Stewart *et al.*, 1968).

Hydrophobicity. Cell-surface hydrophobicity at different stages of growth of a batch culture was tested by partitioning with hexadecane (Rosenberg *et al.*, 1980).

Motility. Motility and longitudinal rotation of the trichomes were investigated after inoculation onto Cooper dishes (Falcon, no. 3009) (Waterbury & Stanier, 1978) containing BG-11 medium, solidified with 0.8% agar. To examine whether the trichomes rotated in response to light intensity, light was applied only through the lids of the Cooper dishes, whose bottoms and sides were protected from light by black paper. The dishes were exposed to light intensities ranging from 5 to $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, at a temperature of 25°C . The orientation of the long axis of the trichomes was determined by measuring the apparent trichome width with a stage micrometer after 2, 4, 6 and 24 h of incubation. The phototactic response of the organism was examined by inoculating the centre of Petri dishes which were covered by black paper in such a way that light entered only from the sides. Results were recorded at regular intervals during a period of 10 d of incubation at light intensities ranging from 10 to $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a temperature of 20°C .

Scanning electron microscopy. Samples were collected on Millipore membrane filters ($0.45 \mu\text{m}$ pore size) and then fixed for 2 h in 4% (v/v) glutaraldehyde in 0.1 M-potassium phosphate buffer, pH 7.2. After fixation, the samples were dehydrated consecutively in ethanol at concentrations of 25–100% (v/v). The 100% ethanol washing was repeated several times to complete dehydration. Subsequently the samples were critical-point dried, gold sputtered and analysed using a Cambridge Instruments S 180 scanning electron microscope.

Transmission electron microscopy. Samples were fixed in 6% (v/v) glutaraldehyde for 2 h, followed by post-fixation with 2% (w/v) osmium tetroxide for 2 h at room temperature. Both fixation steps were performed in 0.1 M-potassium phosphate buffer (pH 7), which was adjusted with sucrose to an osmolarity equivalent to that of the growth medium. Fixed cells were dehydrated successively in ethanol (70–100%, v/v), and subsequently embedded in the epoxy resin of Spurr (1969). Sections (90 nm) were made with a diamond knife on a Reichert & Jung Microtome Ultracut E. The sections were stained with 5% (w/v) uranyl acetate followed by 8% (w/v) lead citrate (Reynolds,

1963), and were examined with a Zeiss electron microscope EM 109 at 50 kV.

Isolation of carbohydrates for X-ray analysis. Water-insoluble carbohydrates were isolated according to a modified procedure of Barclay & Lewin (1985). A batch culture (10 litres), grown to an OD_{750} of 0.5, was harvested by centrifugation and resuspended in 500 ml 0.1 M-potassium phosphate buffer, pH 7.2. The cell suspension was passed three times through a cooled (4°C) French press at 80 MPa and then centrifuged at 20000 g . The pellet was washed twice in 30 ml each of the following solutions: (i) 0.1 M-potassium phosphate buffer, pH 7.2; (ii) distilled water; (iii) diethyl ether/ethanol (1:1, v/v); (iv) acetone. Subsequently the pellet was dried under vacuum and finally suspended in 40 ml 0.1 M-potassium phosphate buffer, pH 7.2. This suspension was treated with $50 \mu\text{g}$ amylase (from *Bacillus subtilis*; specific activity 700–1500 U mg^{-1} ; Sigma) and $50 \mu\text{g}$ amyloglucosidase (from *Aspergillus niger*; specific activity 6 U mg^{-1} ; Boehringer) for 24 h at 20°C . This procedure assured the complete hydrolysis of glycogen. Ice-cold 100% ethanol (100 ml) was added and cell-wall polysaccharides were precipitated for 8 h at 4°C . The precipitate was recovered by centrifugation (20 min, 20000 g , 4°C) and was divided into two parts. One part (fraction A), was analysed without further treatment. The other (fraction B) was resuspended in 15 ml 0.5 M-HCl and hydrolysed for 1 h at 100°C ; the acid-resistant polysaccharides were then precipitated and collected as described for fraction A. Fractions A and B were analysed for cellulose using a Philips PW 1009/80 X-ray apparatus with $\text{Cu}_\text{K}\alpha$ radiation. Powdered Whatman filter paper was used as cellulose reference.

Determination of cellulose and glycogen. A batch culture (500 ml) was centrifuged and the pellet was extracted twice for 1 h in 10 ml methanol (100%) with intermittent centrifugation at 5000 g . The pellet was suspended in 10 ml boiling ethanol (100%) and extracted overnight at 40°C . After centrifugation (5000 g) the pellet was hydrolysed in 5 ml 30% (w/v) KOH for 90 min at 100°C . The hydrolysed samples were centrifuged (10000 g) in order to eliminate cell debris. Polysaccharides in the supernatant were precipitated (2 h, 0°C) after addition of ice-cold ethanol to a final concentration of 75% (v/v). The polysaccharides were collected by centrifugation and dried at 50°C . Glycogen content was determined according to Ernst *et al.* (1984). After complete enzymic digestion of glycogen, the remaining polysaccharides were reprecipitated with ice-cold ethanol (final concentration 75%, v/v), collected by centrifugation and dried at 50°C . The cellulose content of this fraction was quantified according to Updegraff (1969).

Other determinations. Carotenoids were determined by HPLC (Korthals & Steenbergen, 1985). Chlorophyll *a* was determined by the DMF method using a specific absorption coefficient of 72.114 g l^{-1} (Volk & Bishop, 1968). Absorption spectra were recorded on an Aminco DW2000 spectrophotometer and the phycobiliprotein content was estimated according to Tandeau de Marsac & Houmard (1988). Protein was determined by the Folin method (Herbert *et al.*, 1971) using bovine serum albumin as standard. Dry weight was determined on samples washed three times with distilled H_2O and then lyophilized. The cells were examined for the presence of gas vacuoles by the method of Walsby (1973). DNA was extracted and purified by the hydroxyapatite method (Herdman *et al.*, 1979). The mean G+C content was calculated by thermal denaturation according to Marmur & Doty (1962), using calf thymus DNA (Sigma) as reference. Peptidoglycan was measured either as diaminopimelic acid (DAP) or as D-lactic acid in *N*-acetylmuramic acid (Tipper, 1968). A cell suspension (1 litre) from a batch culture was treated (1 h) with 4% (w/v) SDS at 100°C , diluted twofold with distilled H_2O and centrifuged (20 min, 20000 g , 10°C). To completely remove SDS the pellet was washed twelve times with distilled H_2O . DAP was determined qualitatively according to Work (1971). Determination of D-lactic acid in *N*-acetylmuramic acid was

Fig. 1. Scanning electron micrograph of filaments of *C. epipsammum* from a batch culture showing the elliptical shape of the cells. Bar, 6 μ m.

performed by a modification of the method of Tipper (1968). Batch cultures (500 ml) were harvested (5 min, 3800 *g*). The pellets were suspended in 25 ml 0.1 M-Tris/HCl (pH 7) and sonified for 30 min at 4 °C in a Branson Sonifier (50% cycle). The broken cell suspensions were centrifuged (10 min, 3800 *g*) to remove remaining whole cells. The supernatants were then centrifuged at 22000 *g* for 20 min. The resulting pellets and supernatants were hydrolysed at 100 °C with HCl (4 M, final concentration) for 6 h. This treatment was followed by an alkaline hydrolysis with NaOH (4 M, final concentration) for 1 h at 100 °C, after which the samples were filtered through a membrane filter (0.8 μ m pore size, Sartorius). D-Lactate was determined by measuring NAD reduction at 340 nm in the presence of D-lactate dehydrogenase (Boehringer Mannheim).

Table 1. Growth rates of *C. epipsammum* in media of different ionic strength

The organism was grown at 20 °C in batch culture under continuous light at a PFD of 40 μ mol m⁻² s⁻¹, continuously sparged with air (approximately 40 l h⁻¹), at pH 8.0–8.5. Data are expressed as the mean $\pm$ SD of at least four independent determinations.

Growth medium	Growth rate (h ⁻¹)
BG-11 tenfold diluted	0.027 $\pm$ 0.002
BG-11 fivefold diluted	0.037 $\pm$ 0.0015
BG-11 twofold diluted	0.035 $\pm$ 0.0025
BG-11 full strength	0.0295 $\pm$ 0.001
BG-11 + 20 mM-NaCl	0.020 $\pm$ 0.003
BG-11 + 100 mM-NaCl	0.011 $\pm$ 0.002
BG-11 + 200 mM-NaCl	0

Results

Growth characteristics

Crinalium epipsammum appeared to be highly sensitive to elevated concentrations of NaCl (Table 1). Furthermore, although the strain was isolated and cultured in the freshwater medium BG-11, growth was not optimal in this medium. At 20 °C, the highest growth rate in batch culture was obtained in five-fold diluted BG-11 (Table 1). The temperature optimum for growth, determined in full-strength BG-11 medium, was 25 °C, yielding a specific growth rate of 0.044 h⁻¹ in batch culture (data not shown). *C. epipsammum* did not grow in media devoid of combined nitrogen, nor under continuous light or under light : dark cycles. Nitrogenase activity was not detected, even when assayed under strictly anaerobic conditions.

Morphology and ultrastructure

Scanning electron microscopy revealed the typical elliptical shape of the cells of *C. epipsammum* (Fig. 1). The elliptical appearance of the cells was not an artifact due to the preparation of the sample. This was confirmed by thin sections of single cells (Fig. 2) and by light microscopy studies, which revealed a variation in the

Fig. 2. Transmission electron micrograph of a cross-section of a single cell showing its elliptical shape. Bar, 1 μm .

apparent width of the trichomes depending on their orientation (Fig. 3). The narrow sides of the trichomes are 2–2.5 μm , the broad sides 5–7 μm in width. *C. epipsammum* forms straight trichomes that do not taper, and which are composed of short cells (1–1.5 μm in length) (Fig. 3). False or true branching was never observed. The trichome length varied with culture conditions. At light saturation, if sparged with air, the trichomes were less than 400 μm long. Under identical conditions, but exposed to nitrogen- or phosphorus-starvation, the cultures formed even shorter trichomes (less than 200 μm). Under light limitation the trichomes were extremely long (length up to 1000 μm), provided that the cultures were not agitated mechanically or sparged with air. The strain did not differentiate heterocysts or akinetes, even in the absence of combined nitrogen.

Details of the ultrastructure of *C. epipsammum* are shown in Fig. 4(a, b). Thylakoids can be seen as double membranes, mostly peripheral and parallel to the cell wall. Glycogen granules are located between the thylakoid membranes, especially near the cross walls. Polyhedral bodies, probably carboxysomes (Codd & Stewart, 1976), were predominantly observed in the centre of the cytoplasm and occurred in virtually every cell. Gas vacuoles were detected neither by microscopy nor by pressure collapse experiments. The cell envelope of *C. epipsammum* is relatively thick (about 100 nm), due to the rather wide peptidoglycan layer (Fig. 4b). An outer

Fig. 3. Light micrograph (Nomarski interference contrast optics) of filaments of *C. epipsammum* in different orientations showing the variations in apparent width. Note also the lack of differentiation of the end cells. Bar, 20 μm .

membrane is poorly visible (Fig. 4b). The cell envelope contains junctional pores (Halfen & Castenholz, 1971; Guglielmi & Cohen-Bazire, 1982), which are located circumferentially and closely adjacent to either side of the cross-walls. Such pores have been identified in all *Oscillatoria* species examined and represent an important taxonomic property (Rippka, 1988). Cell division of *C. epipsammum* occurs by binary fission in a manner typical of the genus *Oscillatoria* (Drews, 1973): the cytoplasmic membrane forms a cross-septum with simultaneous formation of the peptidoglycan layer. Trichome breakage probably occurs along the circumferential junctional pores adjacent to the cross-septum.

Motility and hydrophobicity

C. epipsammum seems to be permanently non-motile, even when examined after incubation under unidirectional light. It was anticipated that the trichomes would turn their broad side towards light at low light intensities in order to maximize light harvesting, and would orient their narrow side towards light at high light intensities to protect themselves from excessive light. However, experiments with PFD values varying from 5

Fig. 4. Details of the ultrastructure of *C. epipsammum*. (a) Longitudinal section across the narrow side of a filament showing the thylakoids (T) arranged parallel to the cell surface or helically twisted in the cytoplasm; carboxysomes (Cs), junctional pores (JP) and glycogen granules (G). Bar, 0.4 μm ; (b) Cross-section of a single cell demonstrating the thickness (80–100 nm) of the peptidoglycan layer. Bar, 0.2 μm .

Table 2. Cellular components of *C. epipsammum*

The organism was grown at 20 °C in batch cultures in fivefold diluted BG-11 under continuous light at a PFD of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, continuously sparged with air (approximately 40 l h^{-1}), at pH 8.0–8.5. Cells were harvested during exponential growth at $\text{OD}_{750} = 0.5$. Data are expressed as the mean $\pm$ SD of at least three determinations.

Component*	Percentage of dry weight
Protein ^a	20.5 $\pm$ 0.7
Total carbohydrates ^a	47.0 $\pm$ 2.8
Glycogen ^b	22.1 $\pm$ 3.0
Cellulose ^c	21.6 $\pm$ 2.3
Chlorophyll <i>a</i> ^d	1.7 $\pm$ 0.1
<i>N</i> -Acetylmuramic acid ^e	6.6 $\pm$ 0.4

* The constituents were determined according to: a, Herbert *et al.* (1971); b, Ernst *et al.* (1984); c, Updegraff (1969); d, Volk & Bishop (1968); e, Tipper (1968).

to 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ did not indicate any trichome orientation in response to light intensity. Tests for hydrophobicity of the cell surface revealed that all trichomes were hydrophilic, irrespective of the stage of growth of the cultures examined.

Cellular constituents

C. epipsammum grown under continuous illumination in batch culture and harvested at $\text{OD}_{750} = 0.5$ yielded a very high amount of carbohydrates (47% of cell dry weight: Table 2). Enzymic analysis revealed that only part of the carbohydrates (22.1% of cell dry weight) represented glycogen. The remaining material was cellulose, which is probably associated with the cell envelope. X-ray analysis showed the presence of crystals of cellulose II (spacings at 5.68, 4.10, 3.05 and 2.88 Å; 1 Å = 0.1 nm) (Fig. 5a), that were also present in the Whatman filter paper reference sample (Fig. 5b). Cellulose I crystals (spacings at 3.83 and 2.59 Å: compare Figs 5a and 5b) were not present. This X-ray diffraction pattern was only visible after treatment of the polysaccharide fraction with dilute acid, indicating that crystallization was induced by this treatment and is not characteristic of the native material.

The peptidoglycan content of the organism was confirmed by qualitative determination of DAP and quantitative determination of *N*-acetylmuramic acid. On dry weight basis, the cells contained 6.6% *N*-acetylmuramic acid (Table 2). This amount is unusually high for Gram-negative cell walls, but is representative of certain Gram-positive bacteria (Tipper, 1968). Table 3 shows the pigment composition of *C. epipsammum* grown in continuous culture. Phycoerythrin was not present, even after growth in green light. The carotenoid composition

Fig. 5. X-ray diffraction pattern of cell wall polysaccharides after acid treatment (a), and of powdered Whatman filter paper analysed as reference (b). The peaks correspond to the diffraction rings and the numbers represent the crystal spacings (Å; 1 Å = 0.1 nm) as a function of the diameter of the diffraction rings.

of *C. epipsammum* is given in Table 4. The major carotenoids were β -carotene and echinone, but small amounts of zeaxanthine, canthaxanthine and myxoxanthophyll were also found. The relatively high amount of β -carotene detected in *C. epipsammum* is in agreement with the observation of Hertzberg *et al.* (1971) that levels of this carotenoid may be particularly high in cyanobac-

Table 3. Pigment composition of *C. epipsammum*

Cells were grown at 20 °C in BG-11 medium in continuous culture ($D = 0.011 \text{ h}^{-1}$) at pH 8.1, with a light:dark cycle of 8:16 h, under light-limiting conditions (PFD $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Data are expressed as the mean $\pm$ SD of at least three determinations.

Pigment*	Concn [$\mu\text{g (mg protein)}^{-1}$]
Chlorophyll <i>a</i> ^a	29.8 ± 1.6
Allophycocyanin ^b	32.0 ± 9.5
c-Phycocyanin ^b	62.3 ± 12.8
c-Phycocerythrin ^b	0

* The pigments were determined according to: a, Volk & Bishop (1968); b, Tandeau de Marsac & Houmard (1988).

Table 4. Carotenoid composition of *C. epipsammum*

The cultures were grown at 20 °C in BG-11 medium in continuous culture ($D = 0.011$) at pH 8.1, with a light:dark cycle of 8:16 h, under light-limiting conditions (PFD $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Data are from one experiment. The carotenoids were determined by HPLC (Korthals & Steenberg, 1985).

Carotenoid	Percentage (w/w) of total carotenoids
β -Carotene	52
Zeaxanthine	2
Echinone	37
Canthaxanthine	5
Myxoxanthophyll	4

teria isolated from soil. It should be noted that the pigment contents shown in Tables 3 and 4 were determined on samples from continuous cultures grown under light-limiting conditions, in order to maximize pigment synthesis.

Mean DNA base composition

The mean DNA base composition of *C. epipsammum* was determined as 33.9 mol% G + C, which is well below the lower limit of the range of 40–67 mol% G + C found for all filamentous non-heterocystous cyanobacteria studied (Herdman *et al.*, 1979).

Discussion

The organism described here is a member of the *Oscillatoriales* (Anagnostidis & Komarek, 1988; Castenholz, 1989), but differs from all other filamentous non-heterocystous cyanobacteria in culture by having trichomes with elliptical rather than cylindrical cells. It fits best the description of the genus *Crinalium* (Crow, 1927), created for a species encountered as an epiphyte on *Aphanocapsa* colonies, whose trichomes occurred in a hairpin-like configuration (a property responsible for the generic name). The species described by Crow (1927) as *C. endophyticum* has never been cultured. Therefore it is difficult to judge whether or not hairpin formation, which was never observed in the present isolate, is a reliable property for members of this genus. According to Castenholz (1989) the genus *Crinalium* comprises organisms that are characterized by oscillatorian-like trichomes which are elliptical to rectangular in cross-section rather than circular or triradiate. The species described by Crow (1927) possessed cross-walls which, unless stained, were invisible by light microscopy. This property does not apply to *C. epipsammum*, whose cross-walls are readily visible even without staining procedures. Thus, it shares more properties with a second

species, *C. magnum*, described by Fritsch & John (1942), in which cross-walls were also clearly visible and which was placed into the genus *Crinalium* solely on the basis of its elliptical trichomes. However, the description of *C. magnum* by Fritsch & John (1942) does not fit *C. epipsammum* with respect to size. *C. magnum* was reported to possess very broad trichomes (up to 18 µm), whereas the broad side of our strain never exceeds 7 µm. Although the new isolate does not entirely fit either the description of *C. endophyticum* (Crow, 1927) or that of *C. magnum* (Fritsch & John, 1942), we feel confident that the generic assignment to *Crinalium* is justified, and propose to assign the organism to the new species, *C. epipsammum*. However, it should be pointed out that *C. epipsammum* shares certain properties with strains of *Oscillatoria*, i.e. a relatively thick peptidoglycan layer, junctional pores (Guglielmi & Cohen-Bazire, 1982; Rippka, 1988) and the type of cell division and trichome fragmentation (Drews & Weckesser, 1982). However, all *Oscillatoria* species are motile and form cylindrical trichomes which are sometimes surrounded by a thin sheath, whereas *C. epipsammum* is composed of elliptical cells, does not exhibit sheath formation and is non-motile. Furthermore, its G + C content of 33.9 mol% is significantly lower than that described for all organisms assigned to *Oscillatoria* (Herdman *et al.*, 1979), which seems sufficient reason for the separation of the new isolate from the latter genus.

The identification of poly-β-(1,4) glucan in *C. epipsammum* is also novel for cyanobacteria, although its presence has previously been suggested, on the basis of light and electron microscopy, in some heterocystous species (Frey-Wyssling & Stecher, 1954; Tuffery, 1969). In *C. epipsammum* this polymer seems to exist *in vivo* in a non-crystalline form, in contrast to the crystalline form reported for *Acetobacter xylinum* (Aschner & Hestrin, 1946), for the following reason. Filter paper, which was used as the reference for the determination of the β-(1,4)-glucan, is a mixture of cellulose of types I and II. Cellulose of type I, the natural crystalline β-(1,4)-polyglucose of higher plants, gives rise to cellulose of type II by alkaline or acid treatment. A diffraction pattern typical of cellulose type I was lacking in *C. epipsammum*, and that corresponding to its derivative, cellulose type II, was only observed after acid treatment of this polymer. It is not yet clear where the cellulose is located. It seems unlikely to be an extracellular component as in *A. xylinum*, since electron microscopy did not reveal cellulose fibrils external to the cell wall. Therefore it is possible that cellulose is an integral part of the cell wall.

C. epipsammum is a terrestrial cyanobacterium, growing in moving sand, a habitat in which deprivation of light and the availability of water are major problems for the survival of the organism. Both the thick cell wall and

the elliptical shape of the cells may be important adaptations to these extreme environmental conditions. Many terrestrial cyanobacteria possess a sheath. Under conditions where water is a limiting factor, thick mucilaginous sheaths surrounding the trichomes may favour the rapid uptake of water after desiccation (Durrell & Shields, 1961) and help to maintain a relatively humid environment for the organisms (Shephard, 1987). In *C. epipsammum*, in which a sheath was not detected, the thick, presumably cellulose-containing cell wall may serve the same function as a sheath, since pure cellulose has a high capacity to retain water (Updegraff, 1969). Water retention of *C. epipsammum* might also be enhanced as a result of the hydrophilic properties of its cell wall. Thus the structural and biochemical characteristics of this organism may be key elements for its periodic dominance in the extreme environment from which it was isolated.

A formal description of *C. epipsammum* is given below, together with an amended description of the genus *Crinalium*. The original description of this genus by Crow (1927) is poor, and based on uncultured material.

Amended description of the genus *Crinalium* Crow 1927
Cri.na'li.um. L. adj. *crinalis* of hair; Gr. suff. *-ion* diminutive; ML neut. n. *Crinalium* small hairlike (Castenholz, 1989)

Filamentous cyanobacteria (oxygenic photoautotrophs containing chlorophyll *a* as primary photosynthetic pigment and phycobiliproteins as light-harvesting accessory pigments) with trichomes of varying length, composed of cells that are elliptical in cross section; cell division occurs by binary fission in a single plane. The trichomes do not taper, and lack differentiated cells (heterocysts and akinetes).

Crinalium epipsammum sp. nov.

e.pi.psam'mum. Gr. prep. *epi* on, upon; Gr. masc. n. *psammos* sand; ML adj. *epipsammum* on sand

Filamentous cyanobacterium composed of short (1–1.5 µm) elliptical cells that are 5–7 µm in height and 2–2.5 µm in width. End cells are not different in morphology from intercalary cells. The trichomes are non-motile, lack a sheath, may vary in length and do not differentiate heterocysts and akinetes. False or true branching is never observed. Cell division occurs by binary fission; the cytoplasmic membrane forms a cross-septum with simultaneous formation of the peptidoglycan layer. Reproduction occurs by random trichome breakage. The thylakoid membranes are predominantly arranged parallel and close to the periphery of the cells, but may also occur in the centre of the cells, in which case they are curved inwards. Gas vacuoles are not present.

Polyhedral bodies are located in the centre of the cells. Chlorophyll *a* and phycocyanin are the major photosynthetic pigments. β -Carotene and echinone are the major carotenoids. Dinitrogen fixation occurs neither aerobically nor anaerobically. The species is sensitive to elevated concentrations of NaCl, is drought tolerant, and its optimum growth temperature is 25 °C. It was isolated from the surface layers of coarse silica dune sand from the Dutch North Sea coast. The mean DNA base composition is 33.9 mol% G+C (T_m). The type strain has been deposited in the Culture Collection of Göttingen (SAG) under the number 22.89.

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References

- ANAGNOSTIDIS, K. & KOMAREK, J. (1988). Modern approach to the classification system of cyanophytes. 3. Oscillatoriales. *Archives of Hydrobiology Supplement* **80**, 327–472.
- ASCHNER, M. & HESTRIN, S. (1946). Fibrillar structure of cellulose of bacteria and animal origin. *Nature, London* **157**, 659.
- BARCLAY, W. R. & LEWIN, R. A. (1985). Microalgal polysaccharide production for the conditioning of agricultural soils. *Plant and Soil* **88**, 159–169.
- CASTENHOLZ, R. (1989). Subsection III. Order *Oscillatoriales*. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1771–1780. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- CODD, G. A. & STEWART, W. D. P. (1976). Polyhedral bodies and ribulose 1,5-diphosphate carboxylase of the blue-green alga *Anabaena cylindrica*. *Planta* **130**, 323–326.
- CROW, W. B. (1927). *Crinallium*, a new genus of Cyanophyceae, and its bearing on the morphology of the group. *Annals of Botany* **41**, 161–165.
- DE WINDER, B., PLUIS, J., DE REUS, L. & MUR, L. R. (1989). Characterization of a cyanobacterial, algal dune crust in the coastal dunes of The Netherlands. In *Microbial Mats, Physiological Ecology of Benthic Microbial Communities*, pp. 77–83. Edited by Y. Cohen & E. Rosenberg. Washington, DC: American Society for Microbiology.
- DREWS, G. (1973). Fine structure and chemical composition of the cell envelopes. In *The Biology of Blue-green Algae*, pp. 96–116. Edited by N. G. Carr & B. A. Whitton. Oxford: Blackwell Scientific Publications.
- DREWS, G. & WECKESSER, J. (1982). Function, structure and composition of cell walls and external layers. In *The Biology of Cyanobacteria*, pp. 333–359. Edited by N. G. Carr & B. A. Whitton. Oxford: Blackwell Scientific Publications.
- DURRELL, L. A. & SHIELDS, L. M. (1961). Characteristics of soil algae relating to crust formation. *Transactions of the American Microscopy Society* **80**, 73–90.
- ERNST, A., KIRSCHENLOHR, H., DIEZ, J. & BÖGER, P. (1984). Glycogen content and nitrogenase activity in *Anabaena variabilis*. *Archives of Microbiology* **140**, 120–125.
- FREY-WYSSLING, A. & STECHER, H. (1954). Über den Feinbau des Nostoc-Schleimes. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **39**, 515–519.
- FRITSCH, F. E. & JOHN, R. P. (1942). An ecological and taxonomic study of the algae of the British soils. *Annals of Botany, N.S.* **6**, 371–395.
- GUGLIELMI, G. & COHEN-BAZIRE, G. (1982). Structure et distribution des pores et des perforations de l'enveloppe de peptidoglycane chez quelques cyanobactéries. *Protistologica* **18**, 151–165.
- HALFEN, L. N. & CASTENHOLZ, R. W. (1971). Gliding motility in the blue-green alga, *Oscillatoria princeps*. *Journal of Phycology* **7**, 258–260.
- HERBERT, D., PHIPPS, P. J. & STRANGE, R. E. (1971). Chemical analysis of microbial cells. *Methods in Microbiology* **5B**, 209–344.
- HERDMAN, M., JANVIER, M., WATERBURY, J. B., RIPPKA, R., STANIER, R. Y. & MANDEL, M. (1979). Deoxyribonucleic acid base composition of cyanobacteria. *Journal of General Microbiology* **111**, 63–71.
- HERTZBERG, S., LIAANEN-JENSEN, S. & SIEGELMANN, H. W. (1971). The carotenoids of blue green algae. *Phytochemistry* **10**, 3121–3127.
- JUNGERIUS, P. D., VERHEGGEN, A. T. J. & WIGGERS, A. J. (1981). The development of blowouts in 'De Blink' a coastal dune area near Noordwijkerhout, The Netherlands. *Earth Surface Processes and Landforms* **6**, 375–396.
- KORTHALS, H. J. & STEENBERGEN, C. L. M. (1985). Separation and quantification of pigments from natural phototrophic microbial population. *FEMS Microbiology Letters* **31**, 177–181.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.
- RIPPKA, R. (1988). Recognition and identification of cyanobacteria. *Methods in Enzymology* **167**, 28–68.
- RIPPKA, R. & WATERBURY, J. B. (1977). The synthesis of nitrogenase by non-heterocystous cyanobacteria. *FEMS Microbiology Letters* **2**, 83–86.
- RIPPKA, R., DERUELLES, J., WATERBURY, J. B., HERDMAN, M. & STANIER, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**, 1–61.
- ROGER, P. A. & REYNAUD, P. A. (1982). Free living blue-green algae in tropical soils. In *Microbiology of Tropical Soils and Plant Productivity*, pp. 147–168. Edited by Y. R. Dommergues & H. G. Diem. The Hague: Martinus Nijhoff/ Dr W. Junk Publishers.
- ROSENBERG, M., GUTNICK, D. & ROSENBERG, E. (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters* **9**, 29–33.
- SHEPARD, K. L. (1987). Evaporation of water from the mucilage of a gelatinous algal community. *British Phycological Journal* **22**, 181–185.
- SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**, 31–43.
- STEWART, W. D. P., FITZGERALD, G. P. & BURRIS, R. H. (1968). Acetylene reduction by nitrogen fixing blue-green algae. *Archiv für Mikrobiologie* **62**, 336–348.
- TANDEAU DE MARSAC, N. & HOUMARD, J. (1988). Complementary chromatic adaptation: physiological conditions and action spectra. *Methods in Enzymology* **167**, 318–328.
- TIPPER, D. J. (1968). Alkali catalyzed elimination of D-lactic acid and its derivatives and the determination of muramic acid. *Biochemistry* **7**, 1441–1449.
- TUFFERY, A. A. (1969). Light and electron microscopy of the sheath of a blue-green alga. *Journal of General Microbiology* **57**, 41–50.
- UPDEGRAFF, D. M. (1969). Semimicro determination of cellulose in biological materials. *Analytical Biochemistry* **32**, 420–424.

- VAN DEN ANCKER, J. A. M., JUNGERIUS, P. D. & MUR, L. R. (1985). The role of algae in the stabilization of coastal dune blowouts. *Earth Surface Processes and Landforms* **10**, 189–192.
- VOLK, S. L. & BISHOP, N. I. (1968). Photosynthetic efficiency of a phycocyanin-less mutant of *Cyanidium*. *Photochemistry and Photobiology* **8**, 213–221.
- WALSBY, A. E. (1973). A portable apparatus for measuring relative gas vacuolation, the strength of gas vacuoles and turgor pressure in planktonic blue-green algae and bacteria. *Limnology and Oceanography* **18**, 653–658.
- WATERBURY, J. B. & STANIER, R. Y. (1978). Patterns of growth and development in pleurocapsalean cyanobacteria. *Microbiological Reviews* **42**, 2–44.
- WORK, E. (1971). Cell walls. *Methods in Microbiology* **5A**, 361–416.